Advances in High-Throughput Toxicokinetics (HTTK)

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Background

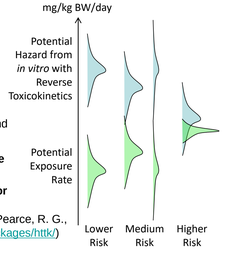
Motivation and Goals

- Data from high throughput *in vitro* screening assays (e.g. Tox21, ToxCast) describe potential **hazard**.
- **Toxicokinetics** (TK) may be used to determine corresponding administered equivalent doses (AED) for comparison to potential intake rate (**exposure**).
- TK may thereby provide a risk based context to *in vitro* toxicity data.
- High throughput (HT) methods and models are necessary to develop TK that are broadly applicable and rapidly deployable; the methods, resulting data, and models collectively encompass high-throughput toxicokinetics (HTTK).

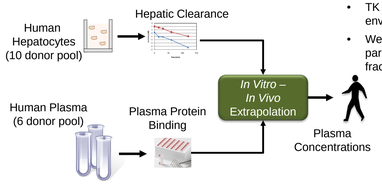
The primary goal of HTTK is to provide a human dose context for bioactive *in vitro* concentrations from high throughput screening assays.

The secondary goal of HTTK is to provide open source data and models for evaluation and use by the broader scientific community.

- models and data are published in the open source R software package *httk* (Pearce, R. G., *et al.* J. of Statistical Software. 79(4), 2017.; https://cran.r-project.org/web/packages/httk/)



Measurement of *In Vitro* TK Parameters



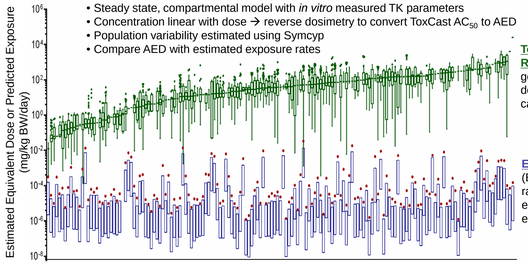
- TK models and data are relatively limited for environmental chemicals.
- We use *in vitro* methods to measure important TK parameters of intrinsic hepatic clearance and the fraction of unbound chemical in plasma.

Rotroff et al. (2010) 35 chemicals
Wetmore et al. (2012) +204 chemicals
Wetmore et al. (2015) +163 chemicals
Wambaugh et al. (in prep.) + ~300 chemicals

Incorporating Dosimetry-Adjusted ToxCast Bioactivity Data with Exposure

Wetmore, B. A., *et al.* Tox. Sci. 148(1), 2015, 121-136

- Steady state, compartmental model with *in vitro* measured TK parameters
- Concentration linear with dose → reverse dosimetry to convert ToxCast AC_{50} to AED
- Population variability estimated using Symcyp
- Compare AED with estimated exposure rates



ToxCast + Reverse Dosimetry generates estimated doses needed to cause bioactivity

Exposure Forecaster (ExpoCast) generates rapid exposure estimates (Wambaugh et al., 2013,2014)

Recent Advances in R Package "httk"

High-throughput physiologically based toxicokinetic model (HT-PBTK):

- Population simulator based on NHANES data
- Calibrating tissue partitioning coefficients
- Evaluating HT-PBTK estimation of *in vivo* TK data in rat
- Measurement of Caco-2 permeability to estimate fraction oral absorption

HT-Population Simulator

Ring, C. L., *et al.* Env. International. 106, 2017, 105-118.

Correlated Monte Carlo sampling of physiological model parameters

Sample quantities from

- Sex
- Race/ethnicity
- Age
- Height
- Weight
- Serum creatinine

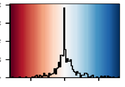
Use equations from literature (McNally *et al.*, 2014) (+ residual marginal variability)

Predict physiological quantities

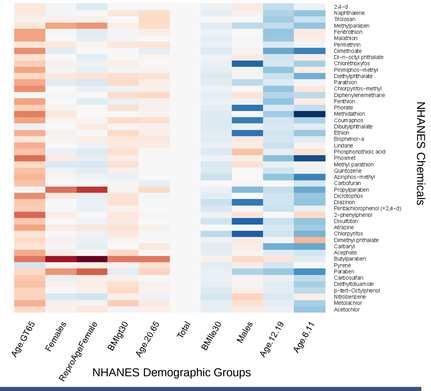
- Tissue masses
- Tissue blood flows
- GFR (kidney function)
- Hepatocellularity

Life-stage and Demographic Specific Predictions

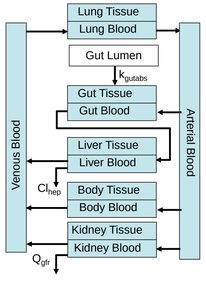
- We use HTTK to calculate margin between bioactivity and exposure for specific populations.
- Most NHANES chemicals do not have traditional PK models (Strope et al., 2018).



- Some demographic groups are potentially more sensitive to exposure to certain environmental chemicals.



Physiologically-Based TK (PBTK)

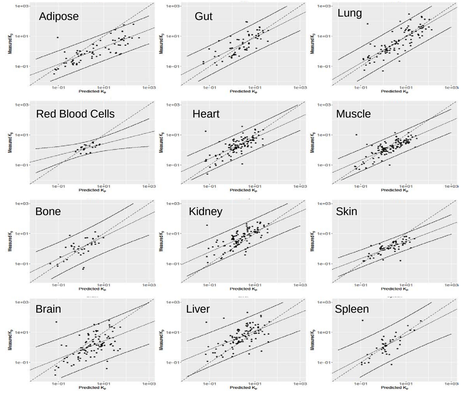


- "httk" includes a generic PBTK model
- Some tissues (e.g. arterial blood) are simple compartments, while others (e.g. kidney) are compound compartments consisting of separate blood and tissue sections with constant partitioning (i.e., tissue specific partition coefficients)
- Exposures are absorbed from reservoirs (gut lumen)
- Some specific tissues (lung, kidney, gut, and liver) are modeled explicitly, others (e.g. fat, brain, bones) are lumped into the "Rest of Body" compartment.
- Blood flows move the chemical throughout the body. The total blood flow to all tissues equals the cardiac output.
- The only ways chemicals "leave" the body are through metabolism (change into a metabolite) in the liver or excretion by glomerular filtration into the proximal tubules of the kidney (which filter into the lumen of the kidney).

Calibrating Tissue Partitioning

Pearce, R. G., *et al.* J. Pharmacokinetics and Pharmacodynamics. 44(6), 2017, 549-565.

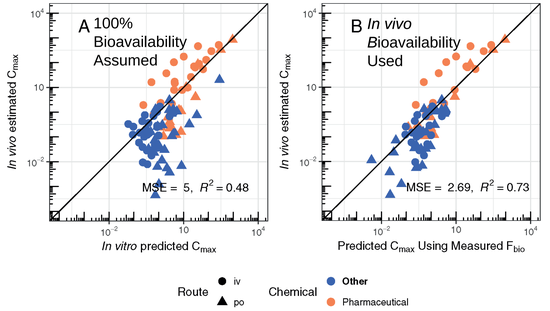
- We analyzed literature measurements of chemical-specific partition coefficients (PC) in rat
  - 945 tissue-specific PC
  - 137 unique chemicals
  - Mostly pharmaceuticals
- We calibrated *in silico* predictors (Schmitt, 2008) to actual performance
- Calibrations were evaluated with human measured volumes of distribution for 498 chemicals from Obach (2008)
  - All pharmaceuticals
- Calibrated tissue partition coefficients provide improved prediction of TK



HT-PBTK Predictions of *In Vivo* TK Data

Wambaugh, J. F., *et al.* Tox. Sci. 163(1), 2018, 152-169.

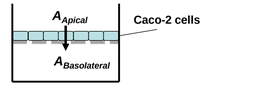
- PBTK predictions can be made for maximum plasma concentration (C_{max}) and for the AUC (time integrated plasma concentration or Area Under the Curve)
- *in vivo* measurements from the literature for various treatments (dose and route) of rat
- Inclusion of oral bioavailability data (Panel B) improves predictions ("httk" assumes default of 100%. Panel A))

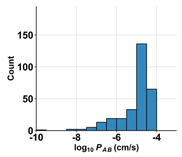


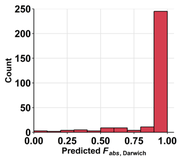
Caco-2 Oral Absorption Data

Honda, G. S., *et al.* In preparation.

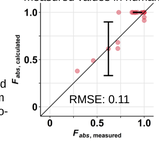
- **Caco-2 cells**, developed from human colon carcinoma cells, form a polarized monolayer that behaves similarly to the human intestinal epithelium.
- Apparent permeability (apical to basolateral; P_{AB}, cm/s) is highly correlated with the fraction of chemical absorbed (F_{abs}) through the gut (Artursson *et al.* 1991).
- We analyze new measurements of P_{AB} for 295 chemicals
- An empirical regression model from literature used to estimate F_{abs} (Darwich *et al.* 2010)
- 83% of chemicals predicted to have F_{abs} > 0.9
- Predicted F_{abs} correlated with values reported in literature (mostly pharmaceuticals, F_{abs} > 0.5).
- Predicted fraction oral bioavailability (F_{bio}) by including first pass hepatic metabolism.
- Calculated F_{bio} poorly correlated with measured values and generally overestimate, metabolism in the intestinal wall not expressed by the Caco-2 cells.



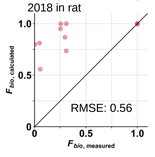




F_{abs} compared with literature measured values in human



Compare F_{bio} with measured values from Wambaugh et al. 2018 in rat



Summary

- Toxicokinetics (TK) provides a bridge between high throughput screening toxicity assays and exposure estimates by predicting tissue concentrations due to external dose
- High Throughput (HTTK) methods developed for pharmaceuticals have been adapted to environmental testing
- R package "httk" freely available on CRAN allows statistical analyses to identify strengths and weaknesses
  - All HTTK models and data made public upon peer-reviewed publication
- Includes one compartment, three compartment (e.g., Wetmore et al.) and generic PBTK model

Ongoing work:

- Development and evaluation of models for additional exposure routes: dermal, inhalation (aerosol and gas)
- A gestational PBTK model

Acknowledgments

- Barbara A. Wetmore (U.S. EPA)
- Cyprotex

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